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IMMUNOFLUORESCENT IDENTIFICATION OF Cl PERFRINGENS IN VARIOUS SECRETIONS

Orvosi hetilap
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Gas edema occurs most frequently when spores of pathogenic clostridium enter lacerations together with particles of soil. Such events occur mainly under conditions of military operations. However, with the development of technology gas edema infections are not rare in trauma connected with the mechanization of agriculture and transportation accidents under peacetime conditions. According to the data of Erno Kienle there were 687 agricultural accidents in 1961 and 1962, in two years, on state farms of Baranya megye, seven of which had fatal outcome. We have no knowledge of how many of these were connected with gas edema infection, but the danger of this always exists in connection with the expansion of mechanized agricultural work (1).

Of the clostridia belonging to the gas edema group Cl. perfringens is the most widespread in nature, and because of this Cl. perfringens must be mentioned among the leading causative agents of gas edema illnesses. The other members of the gas edema group, such as Cl. edematiens, Cl. septicum, etc., are less frequently encountered. In addition to acute traumatic infection, Cl. perfringens is encountered frequently in other pathological processes. According to Weinberg (2)

it plays an important role in gangrenous appendicitis, according to Bergolceva (3) it has been cultured from purulent lung processes, and Williams (4) mentions that Cl. perfringens was detected in an ileus case. It occasionally is cultured from infections of the uterus after childbirth, and abortion. According to a report of Szendi (5) 20 women died of sepsis resulting from illegal abortion during five years (1952-1956) in Bekes Megye. Of these 20 fatal sepsis cases gas edema was the pathological cause in ten (4). Of 78 abortions reported by Douglas et al. (1953) in New York, sepsis mortality caused by Cl. perfringens was recorded in six cases, and also in 73.3 percent of 75 cases of M. Mahn et al. of Santiago (Chile). Six types of Cl. perfringens are known: A. B, C, D, E, and F. Of these, two can cause disease in man. Type A is the most frequently encountered causative agent of gas edema in man; in addition, there is a sub-type that produces alpha toxin, which may also cause food poisoning (7). The work of Bischoff mentions several cases of food poisoning in which Cl. perfringens was found in the bacterial flora cultured from the suspect food (8).

Kemp et al. describe an incident of food poisoning in California in which more than 800 persons became ill. The same serological type Cl. perfringens was cultured from the food and from the feces of the patients (9). Beckmann and Laas described enteritis necroticans cases in 1946. This severe illness with acute course previously had occurred for the first time in Hamburg. Type F of Cl. perfringens is the causative agent of enteritis necroticans. Heim reported three enteritis necroticans patients in 1949 (10). Gas edema cases also may arise from surgical operations and injections. Gy. Vidra and R. Szanto (1954) described gas edema following penicillin injection (11).

It may be seen from the data of the literature that Cl. perfringens may be a disease causative agent in extremely different clinical areas of surgery, obstetrics, internal medicine, etc. Naturally the clinicians frequently turn to the laboratory to confirm the correctness of a diagnosis by culturing the postulated causative agent. If Cl. perfringens causes gas edema this causes no particular difficulty to the surgeon in establishing a diagnosis because this disease has precisely determined and very characteristic signs. In such cases they still refer frequently to the laboratory, and pathologists and justice organizations also request culturing of the causative agent. They send material from a patient (secretion, fluids obtained by puncture tissue removed in the course of an operation, scrapings, etc.) or objects suspected of various infections (bandages, injection drugs, vaccines, etc.) for laboratory examination and bacteriological

identification of clostridia.

The details of anaerobic culturing will not be discussed within the framework of the present article because this belongs to the sphere of responsibility of the bacteriological textbooks. At present we shall merely call attention to the fact that identification of Cl. perfringens by anaerobic culturing and determination of type from any given material very often requires days, and sometimes weeks.

Because of this we turned our attention to the immunofluorescent method, which is relatively simple, rapid, and provides a high degree of specificity. In our work we investigated whether it would be possible to identify Cl. perfringens in material arriving for examination without anaerobic culturing, and with no preparation whatsoever.

We examined 100 samples of the so-called "mixed material" arriving at the department from hospitals and clinics for bacteriological testing, including various fluids obtained by puncture, suppurative secretions, etc. The origin of the 100 samples is shown in the following table.

Name of Material	Number
Facial Cavity Secretion	19
Abcess	3
Punctate	28
Pus	28
Blood	1
Sore Secretion	1
Bronchial Secretion	15
Sternal Punctate	2
Abdominal Punctate	1
Resected Material	2
Total	100

The material was diluted with sodium chloride solution, smeared on an objective slide, fixed with heat, and stained by an indirect fluorescent staining method. The details of the indirect staining method used will not be described because this already has been done in our earlier work (12). Fluoresceinisothiocyanate was used in the staining process. To permit evaluation of the results obtained with the immunofluorescent method the usual bacteriological anaerobic culturing was performed with each sample.

The results of the tests are summarized in the following table.

	Anaerobic Culture Method		Immunofluorescent Method	
	Positive	Negative	Positive	Negative
Facial Cavity Secretion	--	19	2	17
Abcess	--	3	3	--
Punctate	--	28	6	22
Pus	--	28	3	25
Blood	1	--	1	--
Sore Secretion	1	--	1	--
Bronchial Secretion	--	15	1	14
Sternal Punctate	--	2	--	2
Abdominal Punctate	--	1	--	1
Resected Material	2	--	2	--
Total	4	96	19	81

The experimental results show that 4 percent positive results were obtained with the anaerobic culturing method, compared to 19 percent positive results with the immunofluorescent method. Thus the advantage of the immunofluorescent method in demonstrating Cl. perfringens in various secretions not only reduces the test time from several days or weeks to two or three hours, but the method also is shown to be much more sensitive. Of the four positive cases obtained with the culture method the blood and sore secretion samples were obtained from the same patient, who had suffered severe trauma. The two resected material samples also derived from naturally severe infections. It is important to emphasize this to point out the fact that the anaerobic culture procedure is ineffective in the case of a low germ number. Although 28 punctates were tested, the causative agent could not be demonstrated by culturing in any of them, although the causative agent could be identified by culturing in the majority of cases of sepsis, in which massive quantities are present in the blood, and in sore secretion obtained from the site of wounds and infections. In the immunofluorescent method a concentration of 200 bacterial cells per milliliter is sufficient for diagnosis at 10,000,000 dilution and in a mixed culture (13). Positive results were approximately fivefold greater with the immunofluorescent method, which is a valuable and significant achievement in the identification of anaerobic pathological agents.

It may be concluded from the results that in the event that the acids, stains, etc. necessary for the immunofluorescent method are available in the laboratory demonstration of Cl. perfringens by this method is much more advantageous than with the anaerobic culturing method, which requires much work and

time. However, in cases in which the sensitivity of the causative agent to antibiotics or sulfonamid must be tested anaerobic culturing of the causative agent is absolutely necessary. On the basis of present knowledge antibiotic sensitivity cannot be determined by the immunofluorescent method.

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Summary: The authors conducted a study to determine whether Cl. perfringens may be demonstrated in various human secretions (pus, sore secretion, punctate, etc.) by the immunofluorescent method, using no preliminary preparation, because the usual anaerobic culturing method is very lengthy, and may require several days or weeks. ~~They tested~~ 100 different secretions, and for the purpose of comparison the usual bacteriological culture was also performed by the anaerobic method. As a result of the tests, four percent positive indications were obtained with the anaerobic culture method, compared to 19 percent positive results with the immunofluorescent method. Thus the advantage of the immunofluorescent method in demonstration of Cl. perfringens in various secretions consists not only of reducing the test time from several days or weeks to two or three hours, but also of the much greater sensitivity of the method. ~~The authors obtained~~ approximately fivefold greater positive results with the immunofluorescent method, which is a significant achievement in the demonstration of anaerobic germs.

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